constrained pauses and brief anterograde reversals. Mean square displacement analysis and the temperature dependence of transient reversals confirm that motors of opposite polarities (dyneins and kinesins) are both active on the endosomes during retrograde transport. Stochastic multi-motor model simulations show that the biased directionality as well as several statistical metrics of NGF-endosome transport can only be simulated reasonably by assuming that the microtubule-binding affinity of kinesin is down-regulated. Specifically, the simulations suggest that the NGF-endosomes are driven on average by 4-7 active dyneins and 1-3 down-regulated kinesins. These observations are corroborated by the dynamics of endosomes detaching under load in axons; showcasing the cooperativity of multiple dyneins and the subdued activity of kinesins. We discuss the ramifications of our results for intracellular transport regulation, in conjunction with recent studies on cellular cargo in a wide range of motility (bidirectional to unidirectional) regimes.

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Phosphorylation Regulates the Motile Properties of a Mitotic Kinesin

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Saccharomyces cerevisiaes Cin8, a member of the kinesin-5 family of motors, performs important functions in mitotic spindle dynamics such as spindle assembly and anaphase spindle elongation. Resent work has shown that Cin8 is a bidirectional motor and moves *in vitro* towards the minus-end of microtubules (MTs) and changes directionality as a function on ionic strength conditions and MT binding geometry (Gerson-Gurwitz et al., 2011). Previous work from our laboratory had also indicated that Cin8 is differentially phosphorylated during late anaphase at three cyclin-dependent kinase 1 (Cdk1) specific sites located in its motor domain. *In vivo,* this phosphorylation causes Cin8 detachment from the spindles, reduces spindle elongation rate and aids in maintaining proper spindle morphology (Avunie-Masala et al., 2011).
Here, we examined the *in vitro* motile properties of Cin8 by a single-molecule fluorescence motility assay. To study the effect of phosphorylation, we examined the activity of phosphorylation-deficient and phosphorylation-mimic mutant of Cin8. The analysis was done in whole cell extracts as well as on purified Cin8 samples. We found that addition of negative charge in the phospho-mimic mutant weakens the MT-motor interaction and alters the motile properties of Cin8. These results indicate that phosphorylation of Cin8 in the catalytic domain, regulates its motor function.

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Mechano-Chemical Model for the Stepping of Cytoplasmic Dynein

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Cytoplasmic dynein moves processively along microtubules, but it is still unclear how its heads use the energy from ATP hydrolysis, coupled to a linker swing, to achieve directed motion. We present a theoretical model based on a winch mechanism in which the principal direction of the linker stroke is toward the microtubule rather than along it. When mechanically coupling two identical heads, each with postulated elastic properties and a minimal ATPase cycle, the model reproduces stepping with 8-nm steps and directed processivity. Depending on the strength of the interhead connection, the stepping can either be coordinated (tightly coupled heads) or uncoordinated (loosely coupled heads); in the latter case the stepping pattern shows a greater variability. The uncoordinated motor retains a high level of processivity with a reduced product release rate and at the cost of a reduced velocity. The maximum force is largely limited by the loaded motor's processivity, which depends on the product release rates, and can be as high as 6pN in uncoordinated motors. The results of our model show that the winch mechanism is in itself robust and can account for a high stall force and processivity. Its stepping efficiency, however, can be greatly improved by an attractive interaction that leads to the stacking of the two heads.

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Dynactin Functions as Both a Dynamic Tether and Brake during Dynein-Driven Motility

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Dynactin is a large multi-subunit complex that is an important cofactor for cytoplasmic dynein in cellular processes including organelle trafficking and mitosis. Dynactin increases the processivity of dynein and also targets dynein to specific cellular locations. Although the role of dynactin in dynein-mediated processes is well established, the underlying mechanisms involved are unknown. Here, we use single molecule approaches to investigate the contribution of the dynactin subunit p150Glued to dynein motor function. We first characterized the motility of purified GFP-tagged mammalian dynein isolated from a knock-in mouse brain tissue (Zhang et al., 2013). Consistent with previous reports (Mallik et al., 2005; Ross et al., 2006), single molecules of dynein-GFP switch stochastically from processive to diffusive states of motion. We analyzed this motion by a novel algorithm that we developed to parse trajectories into different states and found that dynein is processive 60% of the time. To examine the activation of dynein by dynactin, we investigated the formation and co-migration of a dynein-p150Glued co-complex using dual-color TIRF microscopy. We provide direct evidence that p150Glued is sufficient to recruit and tether dynein to the microtubule and that this recruitment is concentration-dependent; p150Glued increases the on-rate and decreases the off-rate of dynein from microtubules. Single molecule imaging of motility in cell extracts demonstrates that the CAP-Gly domain of dynactin is essential for the decreased detachment rate of the dynein-dynactin complex from the microtubule and also acts as a brake to slow the dynein motor. Consistent with this important role, two neurodegenerative disease-causing mutations in the CAP-Gly domain abrogate these functions in our assays. Together, these observations support a model in which dynactin enhances the initial recruitment of dynein onto microtubules and promotes the sustained engagement of dynein with its cytoskeletal track.

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Towards Simulation of Virtual Cells: The Kinesin-Microtubule Molecular Motor

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Large molecular cargos are transported inside a cell by the walking of a pair of kinesin heads along a microtubule, MT. Despite their biological importance, the mechanism underlying the unidirectionality of kinesin motion is not well understood. Here, the coupling of kinesin's net charge with the large microtubule dipole is conjectured to provide walking directionality, with ATP hydrolysis providing the driving force for kinesin dissociation from the MT. Employing just these two simple assumptions, Brownian Dynamics simulations reproduce multiple experimentally observed features of kinesin walking including the existence of substeps and limping under load, whereby the head that takes the first step walks faster. The latter is caused by hydrodynamic interactions. Hydrodynamic interactions also enhance the relative efficiency of the kinesin motor. We conjecture that electrostatics plays a dominant role in many molecular motors, with charge-dipolar coupling a likely mechanism underlying the directionality of force-generation and electrostatically induced changes concomitant to ATP binding and hydrolysis providing the source of energy.

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Kinesin-2's Role in Intracellular Cargo Transport: Navigating the Complex Microtubule Landscape

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Intracellular cargo transport is a complex and highly regulated process. Molecular motors are responsible for trafficking these cargos to their final destination along a complex microtubule path containing microtubule-associated proteins (MAPs). Cargo regulation has focused intensely on two oppositely moving microtubule motors, dynein and kinesin. However, many cargos contain more than one directionally similar motor, i.e., kinesin-1 and kinesin-2, and the reason for having this combination on a cargo is not well understood. It is equally important to understand how directionally similar teams of motors work together. We have previously shown, in vitro, that kinesin-2's motility is insensitive to a neuronal MAP called Tau, unlike kinesin-1 which is strongly inhibited by Tau. Kinesin-2's longer neck-linker was demonstrated to be essential to its ability to successfully navigate Tau obstacles when compared to kinesin-1's shorter neck-linker. However, the mechanism by which kinesin-2 efficiently navigates microtubule obstacles, such as Tau, is presently unknown. Based on our previous work, we hypothesize that kinesin-2 side-steps to adjacent protofilaments to maneuver around MAPs. To test this, we use single-molecule imaging to track the rotation of microtubules, from the rotation of quantum dots attached to the microtubule surface, in the gliding filament assay to observe torque generation (i.e., side-stepping behavior) in the absence and presence of microtubule obstacles. Understanding kinesin-2's mechanism of navigation on the crowded microtubule surface will elucidate a previously unappreciated and unique contribution to understanding how